

BT COTTON PLANT CULTIVATION AT LOW TEMPERATURE DOES NOT AFFECT HORIZONTAL GENE TRANSFER TO SOIL BACTERIA BUT DECREASES SEED COTTON YIELD

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ABSTRACT

The proportion of cultivable indigenous soil bacteria resistant to kanamycin from *Bt* cotton plant soil was higher than from *nBt* cotton plant soil for cotton crops sown in August. Analysis of 91 *Bt* cotton soil sample total bacteria DNA for the presence of the *nptII* gene was negative. No horizontal gene transfer was detected of the *nptII* gene from *Bt* cotton plant to soil bacteria at crop spacing of 0.65m and 0.35m. The high proportion of indigenous soil bacteria resistant to kanamycin was not due to the transfer of the *nptII* gene from *Bt* cotton crops.

The average temperature in august (32°C) and march (34°C) in eastern Uttar Pradesh did not affect seed germination. For *Bt* cotton sown in August the harvesting time of December and January with an average minimum temperature of 11°C and 8°C respectively was detrimental to seed cotton cultivation. For the Eastern region of Uttar Pradesh *Bt* cotton and *nBt* cotton sown in March showed a 866.4% and 434.7% increase in mean seed cotton yield respectively compared to cotton crops sown in august.

KEYWORDS: *Bt* Cotton, Gene Flow, Horizontal Gene Flow, *nptII*, Seed Cotton Yield

INTRODUCTION

The cultivation of transgenic crops has provided agronomic and economic benefits (Filion 2008). Among the major environmental concerns of cultivating transgenic crops is the unintended spreading of genetic material such as herbicide or antibiotic resistance to microorganisms (Nielsen et al., 2001). Horizontal gene transfer to soil associated microorganisms is the least understood (Filion 2008). Plants effect the abundance, diversity and activity of soil bacteria in their root proximity which affect soil ecology and biogeochemical processes (Pinton et al., 2001). Different bacterial species have been transformed with DNA or tissue material from GM crops under laboratory (Gebhard and Smalla, 1998; Schlüter et al., 1995; Nielsen et al., 2000) and greenhouse conditions (Kay et al., 2002). However these transformations require homologous sequences in the bacterial recipient for integration of exogenous DNA (de Vries and Wackernagel 2002; Demanche et al., 2011).

Horizontal gene transfer from transgenic plant/tree roots to soil microbes has not been observed (Zhang et al., 2005; Dunfield and Germida 2004). Studies on indigenous soil bacteria resistant to ampicillin do not contain the antibiotic *blaTEM* marker gene present in transgenic Bt corn soil samples even when cultivated in the same field over a period of 10 years (Demaneche et al., 2008).

Similarly *Bt* cotton contain antibiotic resistance markers such as the neomycin phosphotransferase II (*nptII*) gene which confers resistance to kanamycin and neomycin. The presence of antibiotic resistance genes in transgenic crops has raised concerns over the possible transfer of antibiotic resistance genes from transgenic crops such as *Bt* cotton to indigenous soil bacteria (Dröge et al 1998). Previous studies have shown that the proportion of indigenous soil bacteria resistant to kanamycin from *Bt* cotton soil samples is higher than from *nBt* cotton samples (Singh et al., 2015). Similarly the proportion of indigenous soil bacteria resistant to kanamycin from *Bt* cotton soil samples sown at a spacing of 0.65m is higher than from spacing of 0.35m (Singh et al., 2015). These results indicate a synergistic effect of *Bt* cotton plants on the growth of kanamycin resistant indigenous bacteria in the immediate vicinity of the *Bt* cotton crop (Singh et al., 2015).

In this manuscript horizontal gene transfer of *nptII* gene from *Bt* cotton crops to soil bacteria was studied by obtaining kanamycin resistant CFU and by *nptII* specific PCR of total bacterial DNA from soil samples. The factors studied were decreased spacing between crops, and; the cultivation of *Bt* cotton was in presence of 50% refuge *nBt* cotton crops sown alternatively, in August. The refuge *nBt* cotton was sown alternatively to prevent resistance development. Simultaneously the seed cotton yield of *Bt* cotton and *nBt* cotton in the west Uttar Pradesh region were recorded and correlation coefficient calculated.

METHODOLOGY

Materials

Bt cotton (cotton 1007- 9810 BG1) and non *Bt* cotton or *nBt* (cotton-Mahesh) seeds were a kind gift from Dr G Garg, Krishidhan, Jalna Maharashtra. *nptII* primers (Kamle et al., 2011) were obtained from Merck and Chromus, India. The sequence of the primers are as follows *nptII* forward primer 5' CTCACCTTGCTCCTGCCGAGA3'; *nptII* reverse primer 5' CGCCTTGA GCCTGGCGAACAG 3'; Taq DNA polymerase and dNTPs were from Merck. *BL21 DE3* containing incorporated *cryIAC* gene and *nptII* was a kind gift from Dr Ananda Kumar, Indian Agriculture Research Institute, New Delhi, India.

Field Trials

The field trials were performed at the horticulture farm of the Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad, Uttar Pradesh. *Bt* and *nBt* seeds were sown in August of 2013. Two plots were maintained at a spacing of 0.65m x 0.65m and 0.35 x 0.35m with alternatively sown *Bt* and *nBt* seeds.

The selected experimental field was harrowed and ploughed with a cultivator and prepared for sowing. Before sowing the field received one full dose of an organic source of field yard manure (FYM); urea as a source of nitrogen at 14gm/m² area; DAP as a source of phosphorus at 8.7gm/m² and MOP as a source of potassium at 6.6gm/m² area. After 5 days seeds were sown in the field at a spacing distance between row to row and plant to plant at 0.65m or 0.35m. While no pesticide was used during the trial, irrigation was at 10 day intervals with hand weeding. Cotton bolls were harvested in May and June. The seed cotton was weighed and the height of the plant measured for all the *Bt* cotton and *nBt* cotton crops from both plot.

Kanamycin Resistant Bacteria from Bt Cotton soil Sample

Soil samples were collected from a depth of 5cm adjacent each plant stem after 4 months and 7 months of sowing cotton seeds to screen for soil bacteria resistant to kanamycin. Briefly 2gm of soil was mixed with 15ml PBS and left at room temperature with shaking for 30 minutes in a sterile falcon tube (Ma et al., 2011). The mud was removed by centrifugation at 600g for 10 minutes and the supernatant containing bacteria was collected for kanamycin screening. 100µl of soil supernatant was used for plating on LB antibiotic plates while from the remaining soil supernatant total bacterial DNA was extracted. 100µl of the supernatant at 1:10 dilution was plated on cycloheximide + Kanamycin (*c+k*) and cycloheximide (*c*) LB plates respectively with both cycloheximide and kanamycin at 50µg/ml. Plates were incubated at 25°C and colonies counted after 1 day for *c* and 2 days for *c+k* plates and recorded as colony forming units/ 1.3mg of soil. When the *c* LB plates contained a lawn of colonies these plates were not counted while their corresponding *c+k* LB plates were counted. The plates containing colonies were stored for possible further analysis.

Total Soil Bacteria DNA Extraction

The soil supernatant from soil samples obtained from above was centrifuged at 1500g for 20 minutes to collect the bacteria (Ma et al., 2011). The total bacterial pellet was resuspended in 400µl of buffer containing 150mM NaCl, 100mM EDTA and 10mM Tris pH 7.9 to which 50µl of 10% SDS was added and vortexed. The bacterial solution was incubated at 60°C for 15 minutes and then cooled to room temperature. To this 450µl of phenol: chloroform (1:2) was added and centrifuged at 10000 rpm for 10 minutes at 4°C. The aqueous layer was gently removed to a fresh tube and 400µl of ice cold 95% ethanol was added. The solution was mixed by inversion and incubated on ice for 30 minutes. The DNA was pelleted at 14,000 rpm for 20 minutes. The supernatant was discarded and the DNA pellet washed in 70% ethanol. The DNA pellet was air dried and resuspended in 50µl TE buffer, quantitated at 260nm and stored at -20°C.

***NptII* Detection in DNA from Soil Bacteria**

NptII detection was by PCR using specific primers (Kamle et al., 2011). The PCR reaction was using Taq DNA polymerase with 1µM primers, 200µM dNTPs and Taq DNA polymerase at 3U/100µl reaction. The reaction condition was 95°C for 10 minutes followed by 40 cycles at 95°C – 30 seconds; 45°C-30 seconds and 72°C – 30 seconds. This was followed by 1 cycle at 72°C for 10 minutes. The positive control for *nptII* gene was *BL21 DE3* containing incorporated *cryIAC* gene. The PCR product was analyzed on a 2% agarose gel.

Correlation Coefficient Calculation

Phenotypic correlations were estimated using the standard procedure (Al-Tabbal and Al-Fraihat, 2012) from the corresponding variance and covariance components using the following equation:

$$r_{pxy} = \frac{\sigma_{pxy}}{\sqrt{\sigma_{px} \times \sigma_{py}}}$$

Where, r_{pxy} = phenotypic correlation coefficient between characters X and Y ; σ_{pxy} the covariance for X and Y and, σ_{px} and σ_{py} the variance for the two characters X and Y.

RESULTS AND DISCUSSIONS

The abiotic factor studied on horizontal gene transfer of *nptII* gene and *Bt* cotton yield was the effect of changing sowing temperature from march to august. Of the many abiotic factors which influence seed germination temperature is the most important (Bradow and Bauer 2010). The effects of suboptimal temperatures are a decrease in seed germination and cotton yield for cotton plants (Bradow and Bauer 2010).

Effect of an abiotic factor such as difference in cultivation temperature on horizontal gene transfer of the *nptII* gene to soil bacteria and seed cotton yield are described. Biotic factors such as cultivation in the presence of 50% refuge *nBt* crops and decreased spacing at sowing of 0.35m on horizontal gene transfer of *nptII* gene to soil bacteria and seed cotton yield are described when sown in August. No pesticide was applied during the field trial since no damage was observed by lepidopteran pests. The presence of the *Bt* crops were sufficient to protect the *nBt* cotton plants sown alternatively i.e., at 50% refuge from lepidopteran damage.

Kanamycin Resistant Bacteria from *Bt* Cotton Soil Sample

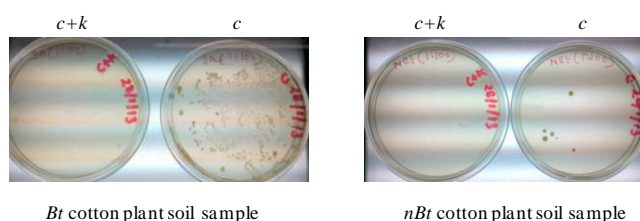


Figure 1: Bacteria from a *Bt* Cotton Plant Soil Sample Spread at 1:10 Dilution on Cycloheximide (c) and Cycloheximide + Kanamycin (c+k) Plates Incubated at 25°C. Colonies were Counted and Recorded as CFU

The colonies on cycloheximide (c) and cycloheximide + kanamycin (c+k) LB plates from soil samples of *Bt* cotton crops were counted and recorded as colony forming units/1.3mg of soil (Figure 1; Table 1). The soil samples from the 0.65 x 0.65m plot on c and c+k plates had a mean CFU of 36 and 10.4 respectively (Table 1). The CFU ranged between 0-164 and 0-109 for c and c+k plates respectively indicating the presence of kanamycin resistant indigenous soil bacteria (Table 1). The proportion of cultivable indigenous soil bacteria resistant to kanamycin was 28.8% (Table 2). When *Bt* cotton was sown during march the proportion of indigenous soil bacteria resistant to kanamycin was 25.29% (Singh et al., 2015; Table 2). The proportion of indigenous soil bacteria resistant to kanamycin was 12.1% higher for *Bt* cotton sown during August than when sown in March.

Table 1: Comparison of Colony Forming Units (CFU) Resistant to Cycloheximide (c) and Cycloheximide + Kanamycin (c+k) from *Bt* Cotton Plant and *nBt* Cotton Plants Soil Samples at 0.65m and 0.3m Spacing. Abbreviations are n: Total Number of Crops; SD: Standard Deviation; CV: Coefficient of Variance. The Soil samples which Gave a Lawn on c LB Plates were Not Counted While there Corresponding c+k LB Plates were

spacing (m)	<i>Bt</i> cotton				<i>nBt</i> cotton			
	0.65		0.3		0.65		0.3	
	c	c+k	c	c+k	c	c+k	c	c+k
n	141	139	36	37	28	28	9	9
mean	36	10.4	45	3	77	10	86.4	4.88
SD	27.8	17.4	59	5.47	42	24.74	27.7	5
CV (%)	77.3	167.7	131	183	54.5	247.4	32	100.8
range	0-164	0-109	3-234	0-30	2-180	0-52	43-128	0-14

The soil samples from *Bt* cotton plants sown at 0.35m spacing on *c* and *c+k* plates had a mean CFU of 45 and 3 respectively (Table 1). The CFU ranged between 3-234 and 0-30 for *c* and *c+k* plates respectively (Table 1). The proportion of indigenous soil bacteria resistant to kanamycin was 6.6% (Table 2). Unlike *Bt* cotton sown at 0.65m, at 0.35m the proportion of indigenous soil bacteria resistant to kanamycin was 186.3% higher for *Bt* cotton sown during March than when sown in August. However the proportion of indigenous soil bacteria resistant to kanamycin was lower when sown at 0.35m than at 0.65m spacing irrespective of time of *Bt* cotton seed sowing (Table 2; Singh et al., 2015). *Bt* cotton when sown at 0.35m spacing showed a lower level of indigenous soil bacteria resistant to kanamycin (Table 1; Singh et al., 2015).

Kanamycin Resistant Bacteria from *nBt* cotton Soil Sample

The soil samples from *nBt* cotton crops sown at 0.65m spacing on *c* and *c+k* plates had a mean CFU of 77 and 10 respectively (Table 1). The CFU ranged between 2-180 and 0-52 for *c* and *c+k* plates respectively indicating the presence of kanamycin resistant indigenous soil bacteria (Table 1). The proportion of indigenous soil bacteria resistant to kanamycin was 12.9% (Table 2). When *nBt* cotton was sown during march the proportion of indigenous soil bacteria resistant to kanamycin was 8.5% (Singh et al., 2015; Table 2). The proportion of indigenous soil bacteria resistant to kanamycin was 34.1% higher for *nBt* cotton sown during August than when sown in March.

The soil samples from *nBt* cotton plants sown at 0.35m spacing on *c* and *c+k* plates had a mean CFU of 86.4 and 4.88 respectively (Table 1). The CFU ranged between 43-128 and 0-14 for *c* and *c+k* plates respectively (Table 1). The proportion of indigenous soil bacteria resistant to kanamycin was 5.6% (Table 2). For *nBt* cotton sown at 0.35m the proportion of indigenous soil bacteria resistant to kanamycin was 37.6% higher for *nBt* cotton sown during August than when sown in March. As seen for *Bt* cotton the proportion of indigenous soil bacteria resistant to kanamycin was lower when sown at 0.35m than at 0.65m spacing irrespective of time of *nBt* cotton seed sowing (Table 2; Singh et al., 2015).

Bt and *nBt* cotton when sown at 0.65m spacing during march and August showed a higher level of indigenous soil bacteria resistant to kanamycin (Table 2; Singh et al., 2015).

Table 2: Proportion of Soil Resistant Bacteria Resistant to Kanamycin from *Bt* and *nBt* Crop Soil Samples Sown in March and August at Crop Spacing of 0.65m and 0.35m

<i>Bt</i> cotton	March*	August
0.65m	25.29	27.7
0.35m	18.9	6.6
<i>nBt</i> cotton	March*	August
0.65m	8.5	12.9
0.35m	3.49	5.6

* Singh et al., 2015

***Npt II* Detection from Total Soil Bacteria DNA Samples of *Bt* Cotton Crops**

Total bacteria DNA was analyzed for the presence of the *nptII* gene by PCR using *nptII* specific primers which amplifies a 215bp fragment and analyzed on a 2% agarose gel. The total number of soil samples analyzed for the presence of the *nptII* gene was 91 for *Bt* cotton samples sown in August (Data not shown). All 91 samples analyzed were negative

for the *nptII* gene (Figure 3; lane 3-8) indicating the complete absence of horizontal gene transfer of *nptII* gene from *Bt* cotton to soil bacteria. Total bacterial DNA of soil samples from nbt cotton crops were used as negative control which did not give the *nptII* gene specific 215bp fragment (data not shown). Similarly no *nptII* transfer was observed for 79 total DNA soil bacteria samples from *Bt* cotton crops sown in March (Singh et al., 2015). These results are consistent with those observed for the 2.3-15.6% cultivable soil bacteria from GM corn (*Zea mays L.*) fields (Ma et al., 2011). None of the kanamycin or neomycin resistant colonies was positive for the *nptII* gene by PCR (Ma et al., 2011). Our results and results on kanamycin and neomycin resistant colonies from GM corn (*Zea mays L.*) trial fields suggests the absence of *nptII* gene in soil bacterial populations (Ma et al., 2011). As observed earlier the prevalence of kanamycin resistant in soil bacteria is therefore not due to gene transfer of *nptII* from *Bt* cotton (Singh et al., 2015). Based on data from total soil bacteria DNA analysis by PCR there is a low possibility of antibiotic transgene transfer from transgenic plants to soil bacterial.

***Bt* and *nBt* Cotton Comparison-August Sowing- 0.65m x 0.65m Spacing**

The comparison of *Bt* cotton and *nBt* cotton at 50% refuge seed sown during August at a spacing of 0.65m shows a mean seed cotton yield of 9.29gm and 6.7gm respectively (Table 3). The mean seed cotton yield of *Bt* cotton was 38% more than *nBt* cotton at 0.65 spacing. A maximum seed cotton yield of 37gm and 19 gm was obtained for *Bt* and *nBt* cotton respectively (Table 3). The mean seed cotton yield of *Bt* cotton and *nBt* cotton at 50% refuge seed sown at a spacing of 0.65m during the month of march gave a mean yield of 69.54gm and 35.83gm respectively (Singh et al., 2015). The lower seed cotton yield for cotton sown in August is due to the low seed cotton harvesting temperature during the month of January and February despite germination in August.

Table 3: Comparison of *Bt* Cotton Plant and *nBt* Cotton Plant Seed Cotton Yield, Plant Height and Number of Cotton Bolls When Sown in August at 0.65m and 0.35m Spacing. Abbreviations are n: Total Number of Crops; SD: Standard Deviation; CV: Coefficient of Variance

	Spacing 0.65m					Spacing 0.35m				
<i>Bt</i> cotton	n	mean	SD	CV (%)	range	n	mean	SD	CV (%)	range
weight (gm)	24	9.29	9.8	106.4	1-37	13	3.4	1.4	40.9	2-6
height (cm)	27	59.9	32	53.4	15-137	13	57	8.9	15.7	39-77
bolls	24	5.7	4.4	77.8	1-6	13	3.76	1.4	36.1	1-6
<i>nBt</i> cotton										
weight (gm)	27	6.7	5.42	56.7	2-19	6	2.74	3	111	1-9
height (cm)	29	43.7	23.2	53	8-96	11	33	12.5	37.8	15-56
bolls	27	5.2	4.5	87.6	1-20	6	1.8	0.84	46	1-3

The mean *Bt* seed cotton yield of the march and august sowing was 69.54gm (Singh et al., 2015) and 9.29 gm (Table 3) respectively with a maximum of 243gm (Singh et al., 2015) and 37gm respectively (Table 3). The mean *nBt* seed cotton yield of the march and august sowing was 35.83 gm (Singh et al., 2015) and 6.7 gm (Table 3) respectively with a maximum of 13gm (Singh et al., 2015) and 19gm respectively (Table 3). *Bt* cotton and *nBt* sown in March gave a 866.4% and 434.7% increase in mean seed cotton yield respectively compared to the august sown crops (Table 4). For the Eastern region of Uttar Pradesh sowing of *Bt* and *nBt* cotton in March give a higher seed cotton yield for both conventional and transgenic cotton (Table 4).

Similarly the mean height of the *Bt* cotton and *nBt* cotton plants when sown in August at 0.65m spacing was 59.9cm and 43.7cm (Table 3). However the mean height of the *Bt* cotton and *nBt* cotton plants when sown in March was 74.14cm and 77.12cm respectively (Singh et al., 2015). The *Bt* and *nBt* crops sown in march were 19.2% and 43% taller than when sown in August.

The mean number of cotton bolls per plant obtained for the *Bt* and *nBt* cotton plants sown in August at 0.65m spacing was 5.7 and 5.2 respectively (Table 3). The maximum number of bolls per plant obtained for *Bt* and *nBt* plants was 6 and 20 respectively (Table 3). However the mean number of bolls of the *Bt* cotton and *nBt* cotton plants when sown in March at 0.65m spacing was 17.31 and 8.65 respectively (Singh et al., 2015). The number of bolls for *Bt* and *nBt* crops sown in march at 0.65m spacing was 203.6% and 66.3% more than when sown in August.

***Bt* and *nBt* Cotton Comparison-August-Sowing -0.35m Spacing**

The comparison of *Bt* cotton and *nBt* cotton at 50% refuge seed sown during August at a spacing of 0.35m gave a mean seed cotton yield of 3.4gm and 2.74gm respectively (Table 3). The mean yield of *Bt* cotton was 24% more than *nBt* cotton at 0.35 spacing. A maximum seed cotton yield of 6gm and 9 gm was obtained for *Bt* and *nBt* cotton respectively (Table 3). The mean seed cotton yield of *Bt* cotton and *nBt* cotton at 50% refuge seed sown at a spacing of 0.35m during the month of march gave a mean yield of 23.5gm and 28.52gm respectively (Singh et al., 2015). The lower seed cotton yield for cotton sown in August is due to the lower spacing of 0.35m between crops and the low seed cotton harvesting temperature during the month of January and February despite germination in August.

The mean *Bt* seed cotton yield of the march and august sowing was 23.5gm (Singh et al., 2015) and 3.4 gm respectively with a maximum of 39gm (Singh et al., 2015) and 6gm respectively (Table 3). The mean *nBt* seed cotton yield of the march and august sowing was 28.52 gm (Singh et al., 2015) and 2.74 gm (Table 3) respectively with a maximum of 36gm (Singh et al., 2015) and 9gm respectively (Table 3). *Bt* cotton and *nBt* sown in March sowing a 591.1% and 940.8% increase in mean seed cotton yield respectively compared to the august sown crops (Table 4). For the Eastern region of Uttar Pradesh sowing of *Bt* and *nBt* cotton in March give a higher seed cotton yield for both conventional and transgenic cotton even at a lower spacing of 0.35m (Table 4). However the maximum yield was obtained for both *Bt* and *nBt* was when sown during the month of March at 0.65m spacing (Table 4).

Table 4: Comparison of *Bt* Cotton plant and *nBt* Seed Cotton Yield Per Plant Sown in March and August at 0.65m and 0.35m Spacing

<i>Bt</i> cotton	0.65m (gm)	0.35m (gm)
March sowing*	69.54	23.5
August sowing	9.29	3.4
<i>nBt</i> cotton		
March sowing*	35.83	28.52
August sowing	6.7	2.74

* Singh et al., 2015

Similarly the mean height of the *Bt* cotton and *nBt* cotton plants when sown in August at 0.35m spacing was 57cm and 33cm (Table 3). However the mean height of the *Bt* cotton and *nBt* cotton plants when sown in March was 52.5cm and 53cm respectively (Singh et al., 2015). The *Bt* and *nBt* crops sown in march was 8.5% shorter and 60% taller than when

sown in August. Despite the shorter height of the *Bt* cotton plant the yield was higher at 0.35m spacing when sown in March than in August.

The mean number of cotton bolls per plant obtained for the *Bt* and *nBt* cotton plants sown in August at 0.35m spacing was 3.76 and 1.8 respectively (Table 3). The maximum number of bolls per plant obtained for *Bt* and *nBt* plants was 6 and 3 respectively (Table 3). However the mean number of bolls of the *Bt* cotton and *nBt* cotton plants when sown in March at 0.35m spacing was 9.4 and 13 respectively (Singh et al., 2015). The number of bolls for *Bt* and *nBt* crops sown in March at 0.35m spacing was 150% and 622.2% more than when sown in August.

Phenotypic correlation coefficient (r) - Analysis for *Bt* cotton Crop Sown in August

The phenotypic correlation coefficient (r) was calculated as described. For *Bt* cotton crops sown in August at 0.65m spacing the seed cotton yield was found positively correlated with the height of the plant with an r value of 0.916 and; with the number of bolls with an r value of 0.979. For *Bt* cotton crops sown in August at 0.35m spacing the seed cotton yield was also found positively correlated with the height of the plant with an r value of 0.941 and; with the number of bolls with a r value of 0.905.

Phenotypic correlation Coefficient (r) - Analysis for *nBt* Cotton Crop Sown in August

The phenotypic correlation coefficient (r) was calculated as described. For *nBt* cotton crops sown in August at 0.65m spacing the seed cotton yield was found positively correlated with the height of the plant with an r value of 0.312 and; with the number of bolls with an r value of 0.822. For *nBt* cotton crops sown in August at 0.35m spacing the seed cotton yield was also found positively correlated with the height of the plant with an r value of 0.872 and; with the number of bolls with a r value of 0.815.

CONCLUSIONS

The proportion of cultivable indigenous soil bacteria resistant to kanamycin was 28.8% and 25.29% when *Bt* cotton was sown in August and March respectively (Table 2). The proportion of indigenous soil bacteria resistant to kanamycin was 12.1% higher for *Bt* cotton sown during August than when sown in March. No *nptII* transgene transfer occurred at 0.65m or 0.35m spacing when sown in both August and March indicating the complete absence of horizontal gene transfer of *nptII* gene to soil bacteria.

In the Eastern region of Uttar Pradesh *Bt* cotton sown in March at 0.65m spacing gave a higher yield of cotton than when sown in August. *Bt* cotton and *nBt* sown in March showed a 866.4% and 434.7% increase in mean seed cotton yield respectively compared to the August sown crops (Table 4). For the Eastern region of Uttar Pradesh sowing of *Bt* and *nBt* cotton in March give a higher seed cotton yield for both conventional and transgenic cotton (Table 4).

Bt cotton plants gave a higher yield than conventional cotton plants indicating *Bt* cotton as a better option for income generation. *Bt* cotton in all instances gave a higher yield of cotton than non-*Bt* cotton irrespective of sowing time or spacing. No pesticide was applied in the field trials reflecting the presence of the *Bt* crops sufficient to protect the *nBt* or refuge cotton plants from *lepidopteran* damage.

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REFERENCES

1. Al-Tabbal, J. A., & Al-Fraihat, A. H. (2012), Genetic variation, heritability, phenotypic and genotypic correlation studies for yield and yield components in promising barley genotypes. *Journal of Agricultural Science*, 4, 193-210
2. Bradow, J. M. & Bauer, P. J. (2010) Germination and seedling development. *Physiology of cotton*, Springer, 48-56.
3. de Vries, J., Meier, P., & Wackernagel, W. (2001) The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. By transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters*, 195, 211–215
4. Demaneche, S., Sanguin, H., Pote, J., Navarro, E., Bernillon, D., Mavingui, P., Wildi, W., Vogel, T. M., & Simonet, P. (2008) Antibiotic-resistant soil bacteria in transgenic plant fields. *PNAS*. 105, 3957–3962
5. Demanèche, S., Monier, J. M., Dugat-Bony, E., & Simonet, P. (2011) Exploration of horizontal gene transfer between transplastomic tobacco and plant-associated bacteria. *FEMS Microbiol. Ecol.* 78, 129-36
6. Dröge, M., Pühler, A., & Selbitschka, W. (1998) Horizontal gene transfer as a biosafety issue: a natural phenomenon of public concern. *J. Biotechnol.* 64, 75-90
7. Dunfield, K. E., & Germida, J. J. (2004) Impact of genetically modified crops on soil- and plant-associated microbial communities. *J. Environ. Qual.* 33, 806-15.
8. Fillion, M. (2008) Do transgenic plants affect rhizobacteria populations. *Microbial. Biotech.* 1, 463-475
9. Gebhard, F., & Smalla, K. (1999) Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology*, 28, 261–272
10. Kamle, S., Kumar, A., & Bhatnagar, R. K. (2011), Development of multiplex and construct specific PCR assay for detection of *cry2Ab* transgene in genetically modified crops and product. *GM Crops*, 2, 74-81
11. Kay, E., Vogel, T. M., Bertolla, F., Nalin, R., & Simonet, P. (2002) In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. *Appl. Environ. Microbiol.* 68, 3345–3351
12. Ma, B. L., Blackshaw, R. E., Roy, J., & He, T. (2011) Investigation on gene transfer from genetically modified corn (*Zea mays* L.) plants to soil bacteria. *Journal of Environmental Science and Health Part B*, 46, 590-599
13. Nielsen, K. M., Elsas, J. D., & Smalla, K. (2000) Transformation of *Acinetobacter* sp. strain BD413 (pFG4DnptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Appl. Environ. Microb.* 66, 1237–1242
14. Nielsen, K. M., Van Elsas, J. D., & Smalla, K. (2001) Dynamics, horizontal transfer and selection of novel DNA in bacterial populations in the phytosphere of transgenic plants. *Ann. Microbiol.* 51, 79–94

15. Pinton, R., Varanini, Z., & Nannipieri, P. (2001) The rhizosphere as a site of biochemical interactions among soil components, plants, and microorganisms. In *The Rhizosphere*. New York: Marcel Dekker, 1–17
16. Schlüter, K., Fütterer, J., & Potrykus, I. (1995) “Horizontal” gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs – if at all – at an extremely low frequency. *Biotechniques*, 13, 1094–1098
17. Singh, R., Prasad, V., Simon, S., & Devasahayam, M. (2015) Effect of spacing and refuge crops on gene transfer of NptII gene from Bt cotton to soil bacteria and on seed cotton yield. *Int J of Geomatics and Geosci*, 5(3), xx-xx. *In press*
18. Zhang, C., Hampp, R., & Nehls, U. (2005) Investigation of horizontal gene transfer in poplar/*Amanita muscaria* ectomycorrhizas. *Environ. Biosafety Res.* 4, 235–242